

**ALLELES OF THE HUMAN MU OPIOID RECEPTOR, DIAGNOSTIC METHODS
USING SAID ALLELES, AND METHODS OF TREATMENT BASED THEREON**
CROSS-REFERENCE TO RELATED APPLICATION

This Application claims priority to provisional application Serial No. 60/212,225, filed June 16, 2000, incorporated herein by reference in its entirety.

GOVERNMENTAL SUPPORT

This invention was made government support under Grant Nos. NIH-NIDA P50-DA05130 and NIH-NIDA K05-DA00049 awarded by the National Institute of Drug Addiction. The Government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates generally to alleles of the human mu opioid receptor gene, along with products derived from such alleles. Also included herein are methods of diagnosing various susceptibilities using such alleles and determining treatment for certain diseases based upon the presence of specific alleles of the human mu opioid receptor gene, and various diseases or disorders related to physiological functions regulated by the hypothalamus pituitary adrenal axis (HPA) or the hypothalamus pituitary gonadal axis (HPG).

BACKGROUND OF THE INVENTION

Opioid drugs have various effects on perception of pain, consciousness, motor control, mood, autonomic function, and can also induce physical dependence. The endogenous opioid system plays an important role in modulating endocrine, cardiovascular, respiratory, gastrointestinal functions, and immune functions. Opioids, either exogenous or endogenous, exert their actions by binding to specific membrane-associated receptors.

Examples of exogenous opioids presently known include, opium, heroin, morphine, codeine, fentanyl, and methadone, to name only a few. Moreover, a family of over 20 endogenous opioid peptides has been identified, wherein the members possess common structural features, including a positive charge juxtaposed with an aromatic ring that is required for interaction

1 with an opioid receptor. It has been determined that most, if not all the endogenous opioid
2 peptides are derived from the proteolytic processing of three precursor proteins, i.e., pro-
3 opiomelanocortin, proenkephalin, and prodynorphin. In addition, a fourth class of endogenous
4 opioids, the endorphins, has been identified (the gene encoding these proteins has not yet been
5 cloned). In the processing of the endogenous opioid precursor proteins, initial cleavages are
6 made by membrane-bound proteases that cut next to pairs of positively charged amino acid
7 residues, and then trimming reactions produce the final endogenous opioids secreted from cells
8 *in vivo*. Different cell types contain different processing enzymes so that, for example
9 proopiomelanocortin can be processed into different endogenous peptides by different cells.
10 For example, in the anterior lobe of the pituitary gland, only corticotropin (ACTH),
11 β -lipotropin, and β -endorphin are produced. Both pro-enkephalin and pro-dynorphin are
12 similarly processed by specific enzymes in specific cells to yield multiple opioid peptides.

13
14 Pharmacological studies have suggested there are numerous classes of opioid receptors which
15 bind to exogenous and endogenous opioids. These classes differ in their affinity for various
16 opioid ligands and in their cellular and organ distribution. Moreover, although the different
17 classes are believed to serve different physiological functions, there is substantial overlap of
18 function, as well as of distribution.

19
20 In particular, there are at least three known types of opioid receptors, mu (μ), delta (δ), and
21 kappa (κ), to which morphine, the enkephalins, and the dynorphins can bind. These three
22 opioid receptor types are the sites of action of opioid ligands producing analgesic effects.
23 However, the type of pain inhibited and the secondary functions vary with each receptor type.
24 The mu receptor is generally regarded as primarily associated with pain relief, and drug or
25 other chemical dependence, i.e., addiction and alcoholism.

26
27 The human mu opioid receptor, which modulates corticotropin releasing hormone, has been
28 isolated and described in PCT Application WO 95/07983 (March 23, 1995) (SEQ ID NO:1) as
29 well as in Chen, Y., Mestek, A., Hurley, J.A., & Yu, L. (1993) *Mol. Pharmacol.* **44**, 8-12,
30 and Wang, et al., *FEBS Letters*, (1994)338:217-222. Furthermore, SEQ ID NO:1 can readily
31 be obtained in GENBANK under accession number L25119. The cDNA therefor contains an

1 open reading frame capable of encoding a protein of 400 amino acid residues with 94 %
2 sequence similarity to the rat mu opioid receptor. Hydropathy analysis of the deduced protein
3 indicates the presence of seven hydrophobic domains, typical of G-protein-coupled receptors.
4 The N-terminus contains five potential N-linked glycosylation sites which remain conserved
5 between the human and the rat mu opioid receptor. A variant in which Asn-40 is changed to
6 Asp (N40D) is reported in GENBANK Accession No. U12569. New polymorphisms G24A
7 (silent), G779A (Arg260His), and G942A (silent) of the mu opioid receptor have been
8 described in co-pending application Serial No. 09/113,426, filed July 10, 1998, and Serial No.
9 09/351,198, filed July 9, 1999, both of which are incorporated herein by reference in their
10 entireties.

11
12 In the body and brain, heroin is biotransformed to morphine, which acts at the mu opioid
13 receptor and results in an euphoric effect and confers the reinforcing properties of the drug and
14 contributes to development of addiction. Heroin addiction can be managed through treatment,
15 primarily methadone maintenance. However, the biological basis of heroin addiction may
16 include diversity of gene structure. Such genetic diversity of the human mu opioid receptor,
17 and the impact of such diversity on receptor function, could contribute to the success or failure
18 of pharmacological management. Similar problems with respect to patient response to
19 pharmacological treatment could occur in most, if not all addictive diseases, such as heroin
20 addiction, alcohol addiction, or cocaine addiction to name only a few, or a combination
21 thereof.

22
23 Moreover, addiction to opioid drugs, especially heroin, is a major social problem in the United
24 States, and throughout the world. For example, recent epidemiological assessments sponsored
25 by the NIH-NIDA and other federal agencies have found that around 2.7 million persons in the
26 United States have used heroin at some time. Moreover, the numbers of "hardcore" long-term
27 heroin addicts (addiction being defined herein as self administration of a regular, multiple,
28 daily dose use of a short-acting opioid, such as heroin, for one year or more, with the
29 development of tolerance, physical dependence and drug-seeking behavior, a definition
30 codified in the Federal guidelines governing pharmacotherapy using long-acting agents such as
31 methadone or LAAM, and used as the minimal requirement for entry into treatment) are now

1 estimated to be approximately one million persons. In addition, it has been estimated that
2 around 24 million persons in the United States have used cocaine for some time, and of that
3 number, approximately one million use cocaine regularly, and at least 600,000-700,000 are
4 cocaine addicts.

5
6 In view of the importance of the human mu opioid receptor in the study of addiction, and the
7 epidemic proportions of drug addiction, especially to heroin, alcohol or cocaine, or a
8 combination thereof, in the United States and throughout the world, and its involvement in the
9 neuroendocrine system, and physiological functions regulated thereby, efforts have been made
10 to investigate whether any polymorphisms in the gene encoding the human mu opioid receptor
11 exist in the population, and whether such polymorphisms result in a phenotype that has an
12 increased or decreased susceptibility towards development of addiction to exogenous opioids,
13 such as heroin, or alcohol, cocaine, or other addictive drugs. For example, in an article
14 entitled "Human mu opioid receptor gene polymorphisms and vulnerability to substance abuse"
15 (Berrettini, W.H., Hoehe, M.R., Ferraro, T.N., DeMaria, P.A., and Gottheil, E., *Addiction*
16 *Biology* 2:303-308 (1997)), two polymorphisms in the human mu opioid receptor gene were
17 reported. One polymorphism (G to T) occurs at nucleotide 175 preceding initiation of
18 translation, and a second coding polymorphism C to T at nucleotide 229 (with respect to
19 transcription initiation) on exon I results in an Ala to Val residue change. However, data taken
20 from a study indicated the C229T polymorphism does not differ in occurrence with statistical
21 significance in addicts relative to non addicts (*idem* at 306). No functional studies were
22 reported.

23
24 It has been further determined that a receptor for both endogenous and exogenous opioids
25 modulates the activity of the hypothalamus pituitary adrenal axis (HPA) and the hypothalamus
26 pituitary gonadal axis (HPG), which effects the neuroendocrine system and its production of
27 signaling compounds that play important roles in regulation of numerous physiological
28 functions. In particular, the neuroendocrine system involves the integration of the neural and
29 endocrine systems of the body, and is responsible for the coordination of numerous bodily
30 functions. An important part of this system is the hypothalamus, a specialized portion of the
31 brain involved in receiving and relaying messages from the central nervous system to other

1 parts of the body. Upon stimulation by chemical signals from the central nervous system, the
2 hypothalamus secretes hypothalamic hormones, such as corticotropin releasing factor (CRF) or
3 hormone and gonadotropin releasing hormone or luteinizing hormone releasing hormone.
4 These factors in turn stimulate the anterior pituitary gland to secrete tropic hormones, or
5 tropins, which are synthesized as relatively long polypeptides, and then are then
6 biotransformed to produce active peptide hormones. Pro-opiomelanocortin, which is processed
7 into several active peptide hormones, including adrenocorticotrophic hormone (ACTH), is an
8 example of a tropic hormone. ACTH stimulates the adrenal cortex to secrete additional
9 hormones, like cortisol, a stress hormone in humans which regulates glucose metabolism, and
10 targets many tissues in the body. In addition, examples of hormones produced by the anterior
11 pituitary gland upon stimulation with gonadotropin releasing hormone include follicle-stimulating
12 hormone and luteinizing hormones. These hormones stimulate the gonads, such as the ovaries
13 and the testes, to secrete androgens, such as testosterone, progesterone, and estrogen, which in
14 turn affect sexual development, sexual behavior, and other reproductive and nonreproductive
15 functions. As a result, the endogenous opioid system plays an important role in modulating
16 endocrine, reproductive, cardiovascular, respiratory, gastrointestinal, immune functions,
17 sexual development and function, as well as a person's response to stress.

18
19 More specifically, in humans, it has been determined that chronic administration of opioids has
20 an inhibitory effect on the HPA axis [McDonald *et al.*, Effect of morphine and nalorphine on
21 plasma hydrocortisone levels in man. *J. Pharmacol. Exp. Ther.* 125:241-247 (1959)]. Basal
22 levels of ACTH and cortisol are significantly disrupted in active heroin addicts: suppression of
23 ACTH and cortisol and abnormal diurnal rhythms of these hormones are found [Kreek,
24 Medical safety and side effects of methadone in tolerant individuals. *JAMA* 223:665-668
25 (1973)]. Basal levels and the diurnal rhythm of ACTH and cortisol, which are disrupted in
26 active heroin addicts, have been shown to become normalized in moderate to high dose, long-
27 term methadone-maintained patients when compared to those of healthy volunteer subjects
28 [Kreek, 1973; Kreek *et al.*, Circadian rhythms and levels of beta-endorphin, ACTH, and
29 cortisol during chronic methadone maintenance treatment in humans. *Life Sci.* 33:409-411
30 (1983); Kreek *et al.*, Prolonged (24 hour) infusion of the opioid antagonist naloxone does not
31 significantly alter plasma levels of cortisol and ACTH in humans. *Proceedings of the 7th*

1 *International Congress on Endocrinology*, Elsevier Science, p. 1170, 1984].

2
3 In healthy volunteers, ACTH and cortisol levels decrease below the basal levels in response to
4 the infusion of β -endorphin indicating feedback of inhibition of pituitary ACTH release or
5 suppression of hypothalamic CRF release by β -endorphin [Taylor *et al.*, Beta-endorphin
6 suppresses adrenocorticotropin and cortisol levels in normal human subjects. *J. Clin.*
7 *Endocrinol. Metab.* 57:592-596 (1983)], and also naloxone (an opioid antagonist) stimulates a
8 rise in serum ACTH and cortisol, suggesting that the HPA axis is under the tonic inhibitory
9 control of endogenous opioids normalized in steady-state chronic methadone-maintained
10 patients; their HPA axis responses to metyrapone-induced stress appear to be no different from
11 that of healthy volunteer subjects [Kreek, 1973; Kreek *et al.*, *Prolonged (24 hour) infusion of*
12 *the opioid antagonist naloxone does not significantly alter plasma levels of cortisol and ACTH*
13 *in humans. Proceedings of the 7th International Congress on Endocrinology* Elsevier Science,
14 p. 1170, 1984].

15
16 Support for the effects of opioids on physiological functions regulated by the HPA and the
17 HPG axes can be found in observations of heroin addicts. More specifically, it has been
18 observed that many heroin addicts are infertile, and in the case of female addicts, their
19 menstrual cycle is dramatically disrupted to the point that they do not ovulate. Furthermore, it
20 has been observed that heroin addicts, and nonaddicted patients taking morphine, become
21 constipated, and that the immune systems of addicts is weakened relative to the immune system
22 of non addicts. However, once therapeutic agents designed to treat addiction, such as
23 methadone, addicts become fertile, are no longer constipated, and have a immune system
24 whose ability to fight foreign bodies is in parity with the immune system of a nonaddict.

25
26 Hence, what is needed is discovery of additional, heretofore unknown polymorphisms of the
27 human mu opioid receptor gene that can be used as genetic markers to map the locus of the
28 human mu opioid receptor gene in the genome.

29
30 What is also needed are the DNA sequences of heretofore unknown isolated nucleic acid
31 molecules which encode human mu opioid receptors, wherein the DNA sequences include a

1 combination of presently known and subsequently discovered polymorphisms of the human mu
2 opioid receptors.

3
4 Furthermore, what is needed is the characterization of the binding properties of heretofore
5 unknown human mu opioid receptors produced from the expression of genes comprising such
6 heretofore unknown polymorphisms of the human mu opioid receptor gene, or combinations of
7 unknown polymorphisms and known polymorphisms.

8
9 Furthermore, what is needed is a characterization of the activity of such unknown human mu
10 opioid receptors produced from the expression of nucleic acid molecules comprising such
11 polymorphisms.

12
13 What is also needed is a correlation between polymorphisms of the human mu opioid receptor
14 gene, and the susceptibility of a subject to addictive diseases, such as heroin addiction, cocaine
15 addiction, or alcohol addiction, to name only a few.

16
17 What is also needed are diagnostic methods to determine a subject's increased or decreased
18 susceptibility to addictive diseases. With the results of such methods, targeted prevention
19 methods, early therapeutic intervention, and improved chronic treatment to opioid addiction
20 can be developed. Physicians armed with the results of such diagnostic methods can
21 determine whether administration to a subject of opioid analgesics is appropriate or whether
22 non-opioid derived analgesics should be administered to the subject. Also, appropriate choice
23 and type of analgesic can be made in treating a subject's pain.

24
25 What is also need are methods of determining a subject's susceptibility to pain and
26 responsibility to analgesics, and using that information when prescribing analgesics to the
27 subject.

28 What is also needed is an ability to determine the binding affinity of the mu opioid receptor to
29 endogenous opioids, such as β -endorphin, and the effect of this binding activity on the
30 neuroendocrine system.

1 The citation of any reference herein should not be construed as an admission that such
2 reference is available as "Prior Art" to the instant application.

3 4 SUMMARY OF THE INVENTION

5 There is provided, in accordance with the present invention, heretofore unknown
6 polymorphisms of the human mu opioid receptor gene, and their use in mapping the locus of
7 the human mu opioid receptor gene, determining susceptibility to addictive diseases,
8 determining susceptibility to pain, and determining a therapeutically effective amount of pain
9 reliever to administer to a subject suffering from pain, diagnosing a disease or disorder in a
10 subject that is related to a physiological function regulated by the HPA or HPG axes of the
11 neuroendocrine system, and selecting an appropriate therapeutic agent and a therapeutically
12 effective amount of such an agent to administer to a subject suffering from a disease or
13 disorder related to a physiological function regulated by the HPA or HPG.

14
15 Hence, the present invention extends to heretofore unknown polymorphisms of the human mu
16 opioid receptor gene that can serve as genetic markers to map the locus of the human mu
17 opioid receptor gene.

18
19 The present invention further extends to DNA sequences of heretofore unknown isolated
20 nucleic acid molecules which encode human mu opioid receptors, wherein the DNA sequences
21 include any combination of presently known polymorphisms and polymorphisms of the human
22 mu opioid receptors discovered by Applicants.

23
24 The present invention further extends to the characterization of the binding properties of
25 heretofore unknown human mu opioid receptors produced from the expression of isolated
26 nucleic acid molecules comprising DNA sequences with such heretofore unknown
27 polymorphisms of the human mu opioid receptor gene, or combinations of unknown
28 polymorphisms and known polymorphisms.

29
30 The present invention further extends to Applicants' discovery that polymorphisms in an allele
31 comprising a DNA sequence of SEQ ID NO:1, such as T67C, T124A, C153T, G174A, and

1 the addition of GGC (a glycine codon) following position 187, hereinafter abbreviated as
2 187INS:GGC, which are described in further detail *infra*, are present in the population. The
3 T67C polymorphism changes serine 23 to a proline (hereinafter abbreviated Ser23Pro), the
4 T124A polymorphism changes serine 42 to a threonine (hereinafter abbreviated Ser42Thr), and
5 the 187INS:GGC adds a glycine residue following glycine 63. The C153T and G174A are
6 silent mutations in the coding region of the mu opioid receptor gene.

7
8 The present invention further extends to diagnostic methods to determine a subject's increased
9 or decreased susceptibility to addictive diseases. With the results of such methods, targeted
10 prevention methods, early therapeutic intervention, and improved chronic treatment to opioid
11 addiction are set forth herein and encompassed by the present invention. In addition, attending
12 medical professionals armed with the results of such diagnostic methods can determine whether
13 administration of opioid analgesics is appropriate or whether non-opioid derived analgesics
14 should be administered to the subject. Furthermore, appropriate choice and type of analgesic
15 to treat a subject's pain can be made. Such determination may be made by identification of any
16 individual or any combination of the above-mentioned polymorphisms, using such non-limiting
17 methods as DNA sequencing, differential hybridization to biological chip arrays such as an
18 oligonucleotide gelpad microchip, or single nucleotide extension (SNE) on chip arrays such as
19 on oligonucleotide gelpad microchips.

20
21 Also, the present invention extends to methods of determining a subject's increased or
22 decreased susceptibility to pain and response to analgesics, and the use of the information in
23 prescribing analgesics to the subject.

24
25 In addition, the present invention extends to methods of diagnosing a disease or disorder in a
26 subject, wherein the disease or disorder is related to a physiological function regulated by the
27 HPA or HPG axes of the neuroendocrine system. Examples of such physiological functions
28 include reproductive or sexual functions, gastrointestinal motility, immune response, and
29 ability to withstand stress.

30
31 Broadly the present invention extends to an isolated variant allele of a human mu opioid

1 receptor gene which can serve as a genetic marker, wherein the predominant or "most
2 common" allele of a human mu opioid receptor gene found in the population comprises a DNA
3 sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA
4 sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A,
5 C153T, G174A, or 187INS:GGC, or any combination thereof.

6
7 Furthermore, the present invention extends to an isolated variant allele of a human mu opioid
8 receptor gene as set forth above, which is detectably labeled. Numerous detectable labels have
9 applications in the present invention, such as radioactive elements, chemicals which fluoresces,
10 or enzymes, to name only a few.

11
12 The present invention further extends to an isolated nucleic acid molecule selectively
13 hybridizable to an isolated variant allele of the human mu opioid receptor gene, wherein the
14 predominant or "most common" allele of a human mu opioid receptor gene found in the
15 population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present
16 invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the
17 variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination
18 thereof.

19
20 Moreover, the present invention extends to an isolated nucleic acid molecule selectively
21 hybridizable to an isolated variant allele of the human mu opioid receptor gene, wherein the
22 predominant or "most common" allele of a human mu opioid receptor gene found in the
23 population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present
24 invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the
25 variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination
26 thereof, wherein the isolated nucleic acid molecule is detectably labeled. Examples of
27 detectable labels that have applications in this embodiment of the present invention are
28 described above.

29
30 In addition, the present invention extends to an isolated variant allele of a human mu opioid
31 receptor gene, wherein the predominant or "most common" allele of the human mu opioid

1 receptor gene encodes a human mu opioid receptor comprising an amino acid sequence of SEQ
2 ID NO:2, and the variant allele of the human mu opioid receptor gene encodes a variant human
3 mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID NO:2,
4 wherein the variation comprises Ser23Pro, Ser42Thr or the addition of a Gly residue following
5 Gly63, or the combination thereof.

6
7 Furthermore, the present invention extends to an isolated nucleic acid molecule selectively
8 hybridizable to an isolated variant allele of a human mu opioid receptor gene of the present
9 invention, wherein the isolated nucleic acid molecule encodes a variant human mu opioid
10 receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the
11 variation comprises Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or
12 the combination thereof.

13
14 Naturally, the present invention extends to a variant human mu opioid receptor comprising an
15 amino acid sequence having a variation in SEQ ID NO:2, wherein the variation comprises
16 Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or the combination
17 thereof.

18
19 Furthermore, the present invention extends to an antibody having as immunogen a variant
20 human mu opioid receptor comprising an amino acid sequence having a variation in SEQ ID
21 NO:2, wherein the variation comprises Ser23Pro, Ser42Thr, or the addition of a Gly residue
22 following Gly63, or both. Such an antibody can be a polyclonal antibody, a monoclonal
23 antibody, or a chimeric antibody. Moreover, an antibody of the present invention can be
24 detectably labeled. Examples of detectable labels which have applications in this embodiment
25 comprises a radioactive element, a chemical which fluoresces, or an enzyme, to name only a
26 few.

27
28 In addition, the present invention extends to cloning vectors that can be used to clone copies of
29 a variant alleles of a human mu opioid receptor gene of the present invention. For example,
30 the present invention extends to a cloning vector comprising an isolated variant allele of a
31 human mu opioid receptor gene and an origin of replication, wherein the predominant or "most

1 common" allele of a human mu opioid receptor gene found in the population comprises a DNA
2 sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA
3 sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A,
4 C153T, G174A, or 187INS:GGC, or any combination thereof.

5
6 In another embodiment, the present invention extends to a cloning vector comprising an
7 isolated nucleic acid molecule selectively hybridizable to an isolated variant allele of a human
8 mu opioid receptor gene, and an origin of replication, wherein the predominant or "most
9 common" allele of a human mu opioid receptor gene found in the population comprises a DNA
10 sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA
11 sequence having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A,
12 C153T, G174A, or 187INS:GGC, or any combination thereof.

13
14 Numerous cloning vectors have applications in the present invention. For example, a cloning
15 vector having applications in the present invention includes *E. coli*, bacteriophages such as
16 lambda derivatives, plasmids such as pBR322 derivatives, and pUC plasmid derivatives such as
17 pGEX vectors or pmal-c or pFLAG, to name only a few.

18
19 Naturally, the present invention extends to expression vectors comprising an isolated variant
20 allele a human mu opioid receptor gene operatively associated with a promoter, wherein the
21 predominant or "most common" allele of a human mu opioid receptor gene found in the
22 population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present
23 invention comprises a DNA sequence having a variation in SEQ ID NO:1, wherein the
24 variation comprises: T67C, T124A, C153T, G174A, or 187INS:GGC, or any combination
25 thereof.

26
27 Furthermore, the present invention extends to an expression vector comprising an isolated
28 nucleic acid molecule selectively hybridizable to an isolated variant allele a human mu opioid
29 receptor gene, wherein the isolated nucleic acid molecule is operatively associated with a
30 promoter. As set forth above, the predominant or "most common" allele of a human mu
31 opioid receptor gene found in the population comprises a DNA sequence of SEQ ID NO:1, and

1 a variant allele of the present invention comprises a DNA sequence having a variation in SEQ
2 ID NO:1, wherein the variation comprises T67C, T124A, C153T, G174A, or 187INS:GGC,
3 or any combination thereof.

4
5 Numerous promoters have applications in an expression vector of the present invention,
6 including but not limited to immediate early promoters of hCMV, early promoters of SV40,
7 early promoters of adenovirus, early promoters of vaccinia, early promoters of polyoma, late
8 promoters of SV40, late promoters of adenovirus, late promoters of vaccinia, late promoters of
9 polyoma, the *lac* the *trp* system, the *TAC* system, the *TRC* system, the major operator and
10 promoter regions of phage lambda, control regions of fd coat protein, 3-phosphoglycerate
11 kinase promoter, acid phosphatase promoter, or promoters of yeast α mating factor, to name
12 only a few.

13
14 In addition, the present invention extends to a unicellular host transformed or transfected with
15 an expression vector of the present invention. Examples of hosts which can be transformed or
16 transfected with an expression vector of the present invention, and have applications in the
17 present invention, include, but are not limited to, *E. coli*, *Pseudomonas*, *Bacillus*,
18 *Streptomyces*, yeast, CHO, R1.1, B-W, L-M, COS1, COS7, BSC1, BSC40, BMT10 or Sf9
19 cells.

20
21 Naturally, the present invention extends to a method of producing a variant human mu opioid
22 receptor comprising an amino acid sequence having a variation in SEQ ID NO:2, wherein the
23 variation comprises Ser23Pro, Ser42Thr, or the addition of a Gly residue following Gly63, or
24 the combination thereof. An example of such a method comprises the steps of culturing a
25 unicellular host transformed or transfected with an expression vector comprising an isolated
26 variant allele a human mu opioid receptor gene, wherein the predominant or "most common"
27 allele of a human mu opioid receptor gene found in the population comprises a DNA sequence
28 of SEQ ID NO:1, and a variant allele of the present invention comprises a DNA sequence
29 having a variation in SEQ ID NO:1, wherein the variation comprises T67C, T124A or
30 187INS:GGC, or the combination thereof, operatively associated with a promoter. The
31 transformed or transfected unicellular host is then cultured under conditions that provide for

1 expression of the variant allele of the human mu opioid receptor gene. The variant human mu
2 opioid receptor produced from such induced expression is then recovered from the unicellular
3 host.

4
5 Another example comprises the steps of culturing a unicellular host transformed or transfected
6 with an expression vector comprising an isolated nucleic acid molecule operatively associated
7 with a promoter, wherein the isolated nucleic acid molecule is selectively hybridizable to a
8 variant allele a human mu opioid receptor gene, and the predominant or "most common" allele
9 of a human mu opioid receptor gene found in the population comprises a DNA sequence of
10 SEQ ID NO:1, and the variant allele comprises a DNA sequence having at least one variation
11 in SEQ ID NO:1, wherein the at least one variation comprises T67C, T124A or 187INS:GGC,
12 or the combination thereof. The transformed or transfected unicellular host is then cultured
13 under conditions that provide for expression of the variant allele of the human mu opioid
14 receptor gene. The variant human opioid receptor produced from such induced expression is
15 then recovered from the unicellular host.

16
17 The invention further extends to altered expression of the mu opioid gene product, and means
18 for detecting the altered expression, as a consequence of the presence of the silent mutations
19 C153T or G174A, or the combination of either or both of the foregoing with any of the other
20 polymorphisms hereindescribed.

21
22 Furthermore, the present invention extends to an isolated variant allele of a human mu opioid
23 receptor gene, wherein the predominant or "most common" allele of the human mu opioid
24 receptor gene comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present
25 invention comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein
26 at least one of the variations is T67C, T124A, C153T, G174A, or 187INS:GGC. The other
27 variation may be any at least one of those described herein or at least one known in the art,
28 such as but not limited to A118G, C17T, G24A, G779A, or G942A.

29
30 The present invention further extends to an isolated variant allele of a human mu opioid
31 receptor gone comprising a DNA sequence having at least two variations in SEQ ID NO:1, as

1 stated above, which is detectably labeled. Examples of detectable labels having applications in
2 this embodiment include, but are not limited to, a radioactive element, a chemical which
3 fluoresces, or an enzyme.

4
5 The present invention further extends to an isolated nucleic acid molecule selectively
6 hybridizable to an isolated variant allele of a human mu opioid receptor gene, wherein the
7 predominant or "most common" allele of the human mu opioid receptor gene comprises a
8 DNA sequence of SEQ ID NO:1, and a variant allele of the present invention comprises a
9 DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one of the
10 variations is T67C, T124A, C153T, G174A, or 187INS:GGC, and the other variation may be
11 any at least one of those described herein or at least one known in the art, such as but not
12 limited to A118G, C17T, G24A, G779A, or G942A.

13
14 Naturally, the present invention extends to a detectably labeled isolated nucleic acid molecule
15 selectively hybridizable to an isolated variant allele of a human mu opioid receptor comprising
16 a DNA sequence having at least two variations in SEQ ID NO:1, wherein at least one of the
17 variations is T67C, T124A, C153T, G174A, or 187INS:GGC, and the other variation may be
18 at least one of those described herein or at least one known in the art, such as but not limited to
19 A118G, C17T, G24A, G779A, or G942A.

20
21 Examples of detectable labels having applications in this embodiment of the invention include,
22 but are not limited to, a radioactive element, a chemical which fluoresces, or an enzyme.

23
24 Furthermore, the present invention extends to an isolated variant allele of a human mu opioid
25 receptor gene comprising a DNA sequence having at least two variations in SEQ ID NO:1, as
26 set forth above, wherein the predominant or "most common" allele of a human mu opioid
27 receptor gene encodes a human mu opioid receptor comprising an amino acid sequence of SEQ
28 ID NO:2, and a variant allele of the present invention encodes a human mu opioid receptor
29 comprising an amino acid having at least two variations in SEQ ID NO:2, wherein the
30 variations comprise Ser23Pro, Ser42Thr or the addition of a Gly residue following Gly63, or
31 both, or at least one of the foregoing or at least one known in the art, such as but not limited to

1 Asn40Asp, Ala6Val, or Arg260His.

2
3 The present invention further extends to an isolated nucleic acid molecule selectively
4 hybridizable to an isolated variant allele of a human mu opioid receptor gene comprising a
5 DNA sequence having at least two variations in SEQ ID NO:1, wherein the variations
6 comprise T67C, T124A, C153T, G174A, or 187INS:GGC, wherein at least one of the
7 variations is T67C, T124A, C153T, G174A, or 187INS:GGC, and the other variation may be
8 any at least one of those described herein or at least one known in the art, such as but not
9 limited to A118G, C17T, G24A, G779A, or G942A, such that the isolated nucleic acid
10 molecule encodes a variant human mu opioid receptor comprising an amino acid sequence
11 having at least two variations in SEQ ID NO:2, wherein the variations comprise at least one of
12 Ser23Pro or conserved variants thereof, Ser42Thr or conserved variants thereof or the addition
13 of a Gly residue following Gly63 or conserved variants thereof, and the other being at least the
14 other of the foregoing or at least one variant known in the art, such as but not limited to
15 Asn40Asp, Ala6Val, or Arg260His.

16
17 Naturally, the present invention extends to a variant human mu opioid receptor comprising an
18 amino acid sequence having at least one variation in SEQ ID NO:2, wherein the variations
19 comprise:

20 Ser23Pro or conserved variants thereof;

21 Ser42Thr or conserved variants thereof;

22 or the addition of a Gly residue following Gly63 or conserved variants thereof.

23
24 Moreover, the present invention extends to an antibody having as an immunogen a human mu
25 opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID
26 NO:2, wherein the variations comprise at least one of Ser23Pro or conserved variants thereof,
27 Ser42Thr or conserved variants thereof or the addition of a Gly residue following Gly63 or
28 conserved variants thereof, and the at least one other being at least one of the other of the
29 foregoing or at least one variant known in the art, such as but not limited to Asn40Asp,
30 Ala6Val, or Arg260His.

1 An antibody of the present invention can be a polyclonal antibody, a monoclonal antibody, or a
2 chimeric antibody. Moreover, an antibody of the present invention can be detectably labeled.
3 Examples of detectable labels having applications in an antibody of the present invention
4 include, but are not limited to, a radioactive element, a chemical which fluoresces, or an
5 enzyme.

6
7 Furthermore, the present invention extends to a cloning vector comprising an isolated variant
8 allele of a human mu opioid receptor gene and an origin of replication, wherein the
9 predominant or "most common" allele of the human mu opioid receptor gene present in the
10 population comprises a DNA sequence of SEQ ID NO:1, and a variant allele of the present
11 invention comprises a DNA sequence having at least two variations in SEQ ID NO:1, wherein
12 at least one the variations is T67C, T124A; C153T; G174A or 187INS:GGC, and the at least
13 one other being one other of the foregoing or at least one known in the art, such as but not
14 limited to A118G, C17T, G24A, G779A, or G942A.

15
16 In addition, the present invention extends to a cloning vector comprising an isolated nucleic
17 acid molecule selectively hybridizable to a variant allele of a human mu opioid receptor and an
18 origin of replication, wherein the variant allele comprises a DNA sequence having at least two
19 variations in SEQ ID NO:1, wherein at least one the variations is T67C, T124A; C153T;
20 G174A or 187INS:GGC, and the at least one other being one other of the foregoing or at least
21 one known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A; and
22 an origin of replication.

23
24 Numerous cloning vectors have applications in this embodiment of the present invention.
25 Examples of such vectors include, but are not limited to, *E. coli*, bacteriophages, such as
26 lambda derivatives, plasmids such as pBR322 derivatives, and pUC plasmid derivatives such as
27 pGEX vectors or pmal-c or pFLAG, to name only a few.

28
29 Naturally, the present invention extends to an expression vector comprising an isolated variant
30 allele of a human mu opioid receptor gene operatively associated with a promoter, wherein
31 such an isolated variant allele comprises a DNA sequence having at least two variations in SEQ

1 ID NO:1, wherein at least one the variations is T67C; T124A; C153T; G174A or
2 187INS:GGC, and the at least one other being one other of the foregoing or at least one variant
3 known in the art, such as but not limited to A118G, C17T, G24A, G779A, or G942A.
4

5 In addition, the present invention extends to an expression vector comprising an isolated
6 nucleic acid molecule operatively associated with a promoter, wherein the isolated nucleic acid
7 molecule is selectively hybridizable to an isolated variant allele of a human mu opioid receptor
8 gene comprising a DNA sequence having at least two variations in SEQ ID NO:1, wherein at
9 least one the variations is T67C; T124A; C153T; G174A or 187INS:GGC, and the at least one
10 other variation being one other of the foregoing or at least one variant known in the art, such
11 as but not limited to A118G, C17T, G24A, G779A, or G942A.
12

13 Numerous promoters are available and have applications in an expression vector of the present
14 invention. Examples of promoters having applications include, but are not limited to
15 immediate early promoters of hCMV, early promoters of SV40, early promoters of
16 adenovirus, early promoters of vaccinia, early promoters of polyoma, late promoters of SV40,
17 late promoters of adenovirus, late promoters of vaccinia, late promoters of polyoma, the *lac*
18 the *trp* system, the *TAC* system, the *TRC* system, the major operator and promoter regions of
19 phage lambda, control regions of fd coat protein, 3-phosphoglycerate kinase promoter, acid
20 phosphatase promoter, or promoters of yeast α mating factor, to name only a few.
21

22 Naturally, the present invention extends to a unicellular host transformed or transfected with an
23 expression vector of the present invention. Examples of unicellular hosts having applications
24 in an embodiment of the present invention include, but are not limited to, *E. coli*,
25 Pseudonomas, Bacillus, Streptomyces, yeast, WHO, R1.1, B-W, L-M, COS1, COS7, BSC1,
26 BSC40, BMT10 or Sf9 cells.
27

28 In another embodiment, the present invention extends to a method for producing a human mu
29 opioid receptor comprising an amino acid sequence having at least two variations in SEQ ID
30 NO:2, wherein the variations comprise at least one of Ser23Pro or conserved variants thereof,
31 Ser42Thr or conserved variants thereof or the addition of a Gly residue following Gly63, or

1 conserved variants thereof; and the at least one other being the other of the foregoing or at
2 least one variant known in the art, such as but not limited to Asn40Asp, Ala6Val, or
3 Arg260His.

4
5 More specifically, an example of a method for producing such a human mu opioid receptor
6 comprises the steps of culturing a unicellular host transformed or transfected with an
7 expression vector comprising an isolated variant allele of a human mu opioid receptor gene
8 operatively associated with a promoter, wherein the variant allele comprises a DNA sequence
9 having at least two variations in SEQ ID NO:1, wherein at least one the variations is T67C;
10 T124A; C153T; G174A or 187INS:GGC, and the at least one other variation being one other
11 of the foregoing or at least one variant known in the art, such as but not limited to A118G,
12 C17T, G24A, G779A, or G942A; under conditions that provide for expression of the isolated
13 variant allele of a human mu opioid receptor gene. After expression, a variant human mu
14 opioid receptor is recovered from the unicellular host.

15
16 In another example, a method for producing a human mu opioid receptor of the present
17 invention comprises the steps of culturing a unicellular host transformed or transfected with an
18 expression vector comprising an isolated nucleic acid molecule operatively associated with a
19 promoter, wherein the isolated nucleic acid molecule is selectively hybridizable to an isolated
20 variant allele of a human mu opioid receptor gene comprising a DNA sequence having at least
21 two variations in SEQ ID NO:1, wherein at least one the variations is T67C; T124A; C153T;
22 G174A or 187INS:GGC, and the at least one other variation being one other of the foregoing
23 or at least one variant known in the art, such as but not limited to A118G, C17T, G24A,
24 G779A, or G942A, under conditions that provide for expression of the isolated nucleic acid
25 molecule. The variant human mu opioid receptor produced from the expression is then
26 recovered from the unicellular host.

27
28 The present invention also embraces functional variants of the mu opioid receptor as a
29 consequence of the presence of at least one of the polymorphisms described herein, either as
30 the only polymorphism as compared to the wild-type gene or in combination with any number
31 of other polymorphisms, including the others described herein or those known in the art. The

1 invention is further directed to methods for detecting altered gene product structure, activity or
2 function, said altered structure, activity or function resulting from the presence of at least one
3 of the polymorphisms described herein.

4
5 Accordingly, the present invention extends to a method for determining a susceptibility in a
6 subject to at least one addictive disease, comprising the steps of removing a bodily sample
7 comprising a first and second allele of a human mu opioid receptor gene from the subject, and
8 determining whether the first allele comprises a human mu opioid receptor gene comprising a
9 DNA sequence having at least one variation in SEQ ID NO:1, wherein the variation comprises:
10 T67C; T124A; or 187INS:GGC.

11
12 The present of at least one of these variations in the human mu opioid receptor gene of the first
13 allele is expected to be indicative of the subject's susceptibility to at least one addictive disease
14 relative to the susceptibility of a standard to at least one addictive disease, wherein the standard
15 comprises a first allele comprising a human mu opioid receptor gene having a DNA sequence
16 of SEQ ID NO:1.

17
18 Another embodiment of the method for determining a susceptibility in the subject to at least one
19 addictive disease, as described above, comprises the further step of determining whether the
20 second allele of the bodily sample of the subject comprises a human mu opioid receptor gene
21 comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the
22 variations comprise T67C, T124A or 187INS:GGC.

23
24 The presence of at least one variation the second allele of the bodily sample is expected to be
25 indicative of the subject's susceptibility to at least one addictive disease relative to a standard in
26 which both alleles of a human mu opioid receptor gene comprise a DNA sequence of SEQ ID
27 NO:1.

28
29 In another embodiment, the present invention extends to a method for determining a
30 susceptibility to at least one addictive disease in a subject relative to susceptibility to at least
31 one addictive disease in a standard, involving the detection of variations in the human mu

1 opioid receptor itself, and particularly, determining whether a variant human mu opioid
2 receptor is present in a bodily sample from a subject. Such a method comprises the steps of
3 removing a bodily sample comprising a human mu opioid receptor from the subject, and
4 determining whether the human mu opioid receptor present in the sample is a variant human
5 mu opioid receptor of the invention, wherein the variant human mu opioid receptor comprises
6 an amino acid sequence having at least one variation in SEQ ID NO:2, wherein the at least one
7 variation comprises: Ser23Pro, Ser42Thr or conserved variants thereof; or the addition of a
8 Gly residue following Gly63 or conserved variants thereof, the presence of at least one
9 variation is expected to be indicative of the subject's susceptibility to at least one addictive
10 disease relative to susceptibility to at least one addictive disease in a standard, wherein the
11 human mu opioid receptor of the standard comprises an amino acid sequence of SEQ ID NO:2.

12
13 As explained above, at least one addictive disease includes, but is not limited to, opioid
14 addiction, cocaine addiction or addiction to other psychostimulants, nicotine addiction,
15 barbiturate or sedative hypnotic addiction, anxiolytic addiction, or alcohol addiction.

16
17 Furthermore, the present invention extends to a method for determining a susceptibility to pain
18 in a subject relative to susceptibility to pain in a standard, comprising the steps of removing a
19 bodily sample comprising a first and second allele of a human mu opioid receptor gene from
20 the subject, and determining whether the first allele comprises a human mu opioid receptor
21 gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the
22 variation comprises: T67C, T124A or 187INS:GGC. The presence of at least one variation in
23 the human mu opioid receptor gene of the first allele is expected to be indicative of a decreased
24 or increased susceptibility to pain in the subject relative to susceptibility to pain in the standard,
25 wherein the first allele of the standard comprises a human mu opioid receptor gene comprising
26 a DNA sequence of SEQ ID NO:1.

27
28 Moreover, a method for determining a susceptibility to pain in a subject may further comprise
29 the step of determining whether the second allele comprises a human mu opioid receptor gene
30 comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the
31 variation comprises: T67C, T124A or 187INS:GGC. The presence of the at least one variation

1 in the human mu opioid receptor gene of the second allele of the bodily sample from the
2 subject is expected to be indicative of an increased or decreased susceptibility to pain in the
3 subject relative to the susceptibility to pain in the standard, wherein the second allele in the
4 standard comprises a human mu opioid receptor gene comprising a DNA sequence of SEQ ID
5 NO:1.

6
7 In another embodiment, the present invention extends to a method for determining a
8 susceptibility to pain in a subject relative to susceptibility to pain in a standard by examining a
9 bodily sample taken from the subject for the presence of a variant human mu opioid receptor.
10 Such a method comprises the steps of removing a bodily sample comprising a human mu opioid
11 receptor from the subject, and determining whether the human mu opioid receptor present in
12 the sample is a variant human mu opioid receptor of the invention, i.e., comprises an amino
13 acid sequence having at least one variation in SEQ ID NO:2, wherein the variation comprises:

14 Ser23Pro or conserved variants thereof;

15 Ser42Thr or conserved variants thereof; or

16 addition of a Gly residue following Gly63 or conserved variants thereof,

17 such that the presence of at least one variation is expected to be indicative of the subject's
18 susceptibility to pain relative to susceptibility to pain in the standard, wherein the human mu
19 opioid receptor of the standard comprises an amino acid sequence of SEQ ID NO:2.

20
21 Once a susceptibility to pain in the subject has been determined, it is possible for attending
22 medical professionals treating the subject to administer to an appropriate, or therapeutically
23 effective amount of pain reliever in order to induce analgesia in the subject. Administration of
24 such an amount is important to the subject because, should an inappropriate amount of pain
25 reliever be administered, the subject may not experience analgesia, and may be exposed to
26 potentially deleterious side effects of the pain reliever, such as induction of addiction to the
27 pain reliever, brain damage, or death.

28
29 Consequently, the present invention extends to a method for determining a therapeutically
30 effective amount of pain reliever to administer to a subject in order to induce analgesia in the
31 subject relative to a therapeutically effective amount of the pain reliever to administer to a

1 standard in order to induce analgesia in the standard, wherein the method comprises
2 determining a susceptibility to pain in the subject relative to susceptibility to pain in the
3 standard. The susceptibility of pain in the subject is expected to be indicative of the
4 therapeutically effective amount of the pain reliever to administer to the subject to induce
5 analgesia in the subject relative to the amount of the pain reliever to administer to the standard
6 to induce analgesia in the standard.

7
8 Hence, the present invention extends to a method for determining a therapeutically effective
9 amount of pain reliever to administer to a subject in order to induce analgesia in the subject
10 relative to a therapeutically effective amount of the pain reliever to administer to a standard in
11 order to induce analgesia in the standard wherein the method comprises the steps of removing a
12 bodily sample comprising a first and second allele of a human mu opioid receptor gene from
13 the subject, and determining whether the first allele comprises a human mu opioid receptor
14 gene comprising a DNA sequence having at least one variation in SEQ ID NO:1, wherein the
15 at least one variation comprises: T67C, T124A or 187INS:GGC. The presence of at least one
16 variation in the human mu opioid receptor gene of the first allele from the bodily sample is
17 expected to be indicative of the therapeutically effective amount of pain reliever to administer
18 to the subject to induce analgesia in the subject relative to the therapeutically effective amount
19 of pain reliever to administer to the standard to induce analgesia in the standard, wherein the
20 standard comprises a first allele comprising a human mu opioid receptor gene comprising a
21 DNA sequence of SEQ ID NO:1.

22
23 Moreover, the present invention further extends to a method for determining a therapeutically
24 effective amount of pain reliever to administer to a subject in order to induce analgesia in the
25 subject relative to a therapeutically effective amount of pain reliever to administer to a standard
26 to induce analgesia therein, further comprising the steps of removing a bodily sample
27 comprising a first and second allele comprising a human mu opioid receptor gene from the
28 subject, and determining whether the second allele of the bodily sample comprises a human mu
29 opioid receptor gene comprising a DNA sequence comprising at least one variation in SEQ ID
30 NO:1, wherein the at least one variation comprises: T67C, T124A or 187INS:GGC. The
31 presence of at least one variation in the human mu opioid receptor gene of the first and/or

1 second allele of the bodily sample is expected to be indicative of the therapeutically effective
2 amount of pain reliever to administer to the subject to induce analgesia therein relative to the
3 amount of pain reliever to administer to a standard to induce analgesia therein, wherein the first
4 and second alleles of the standard comprise a human mu opioid receptor gene comprising a
5 DNA sequence of SEQ ID NO:1.

6
7 In another embodiment, the present invention extends to determining a therapeutically effective
8 amount of pain reliever to administer to a subject in order to induce analgesia in the subject, by
9 examining a bodily sample from a subject for the presence of a variant human mu opioid
10 receptor comprising an amino acid sequence having a variation in SEQ ID NO:2. More
11 specifically, the present invention extends to a method for determining a therapeutically
12 effective amount of pain reliever to administer to a subject in order to induce analgesia in the
13 subject, relative to a therapeutically effective amount of pain reliever to administer to a
14 standard in order to induce analgesia in the standard, comprising the steps of removing a bodily
15 sample comprising a human mu opioid receptor from the subject, and determining whether the
16 human mu opioid receptor present in the sample comprises an amino acid sequence having at
17 least one variation in SEQ ID NO:2, wherein the variation comprises:

18 Ser23Pro or conserved variants thereof;

19 Ser42Thr or conserved variants thereof; or

20 addition of a Gly residue following Gly63 or conserved variants thereof,

21 such that the presence of at least one variation is expected to be indicative of the therapeutically
22 effective amount of pain reliever to administer to the subject to induce analgesia therein relative
23 to the therapeutically effective amount of pain reliever to administer to induce analgesia in the
24 standard, wherein the human mu opioid receptor of the standard comprises an amino acid
25 sequence of SEQ ID NO:2.

26
27 Examples of pain relievers having applications in this embodiment of the present invention
28 include, but are not limited to, morphine, codeine, dihydromorphan, meperidine, methadone,
29 fentanyl and its congeners, butorphenol, nalbuphine, LAAM, or propoxyphene, to name only a
30 few.

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1 Furthermore, the present invention extends to a method for determining a therapeutically
2 effective amount of a therapeutic agent for treating at least one addictive disease to administer
3 to a subject suffering from at least one addictive disease, relative to a therapeutically effective
4 amount of the therapeutic agent to administer to a standard suffering from the at least one
5 addictive disease. As a result, the dosage of therapeutic agent administered to an addict can be
6 "tailored" to the addict's needs based upon the addict's genotype. An example of such a
7 method comprises the steps of removing a bodily sample from the subject, wherein the bodily
8 sample comprises a first and second allele of the human mu opioid receptor gene, and
9 determining whether the first allele comprises a DNA sequence having at least one variation in
10 SEQ ID NO:1, wherein the variation comprises: T67C, T124A or 187INS:GGC. The
11 presence of the at least one variation in the human mu opioid receptor gene of the first allele in
12 the bodily sample from the subject is related to the therapeutically effective amount of
13 therapeutic agent to administer to the subject to treat the subject's at least one addictive disease,
14 relative to the therapeutically effective amount of the therapeutic agent to administer to the
15 standard suffering from the at least one addictive disease, wherein the first and second allele of
16 the standard comprise a human mu opioid receptor gene comprising a DNA sequence of SEQ
17 ID NO:1.

18
19 Furthermore, a method for determining a therapeutically effective amount of therapeutic agent
20 to administer to a subject suffering from at least one addictive disease may further comprise an
21 additional step of determining whether the second allele of the bodily sample taken from the
22 subject comprises a human mu opioid receptor gene comprises a DNA sequence having at least
23 one variation in SEQ ID NO:1, wherein the at least one variation comprises: T67C, T124A or
24 187INS:GGC. Such a variation in the first and/or second allele of the bodily sample is
25 expected to be indicative of the therapeutically effective amount of the therapeutic agent to
26 administer to the subject to treat the at least one addictive disease of the subject relative to the
27 therapeutically effective amount of the therapeutic agent to administer to the standard suffering
28 from the at least one addictive disease.

29
30 In another embodiment, the present invention extends to determining a therapeutically effective
31 amount of a therapeutic agent for treating at least one addictive disease to administer to a